

# Do Metabolite Transport Processes Limit Photosynthesis?<sup>1</sup>

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Metabolite transport proteins occupy key positions in the metabolic networks of highly compartmentalized eukaryotic cells. In such cells, at least one and frequently more than one membrane delineate organelles from the cytosol. Thereby compartments with different reaction milieus are created that are characterized by low pH or high-energy status, or by highly reductive or oxidative conditions, as compared to the cytosol. The membranes bounding these compartments act as effective diffusion barriers to most organic metabolites and inorganic ions. However, cellular metabolic networks frequently extend over several compartments. Hence, substrates, intermediates, and products of pathways need to pass the bounding membranes in a controlled manner that retains the compartment-specific conditions while allowing the passage of defined metabolites. Transport proteins that are embedded in the membrane enable the passage of metabolites and thereby connect metabolic networks beyond organellar boundaries (Linka and Weber, 2010).

Transport proteins can be broadly classified into three groups: channels or pores, primary active transporters, and secondary active transporters, respectively (Heldt, 1999). Channels or pores permit the diffusion of molecules along a concentration gradient or electrochemical potential. Since diffusion of solutes through pores and channels does not involve binding of the substrate to the channel protein but its passage through the hydrophilic channel pore, diffusion occurs very fast, up to  $10^6$  molecules per second (Heldt, 1999). In contrast, carrier proteins, similar to enzymes, bind their substrates and undergo a conformational change upon binding and transport. Hence, transport processes mediated by carrier proteins are several orders of magnitude slower than those mediated by channels, ranging between 10 and several thousand molecules per second (Heldt, 1999). Primary active transporters split energy-rich bonds such as those in ATP or inorganic pyrophosphate to transport metabolites or ions against a concentration gradient. Secondary active transport proteins act as either symporters or antiporters, respectively. That is, they transport one molecule against its concentration gradient, whereas another is either transported in the same (symport) or the oppo-

site direction (antiport) along its concentration gradient. This cotransport mode is mandatory, meaning that under physiological conditions, transport of one molecule cannot occur without the other. The larger, favorable change in free energy of one substrate drives the flux of the second molecule against its electrochemical potential difference. Especially for secondary transporters, it is important to consider the net transport activity, which can be calculated from the symport by addition and from the antiport by subtraction. The majority of transport proteins involved in transporting metabolites resulting from photosynthesis are of the secondary active transporter type. Since secondary transporters, as outlined above, have low turnover numbers, relatively large amounts of such proteins are required if large fluxes have to be accommodated.

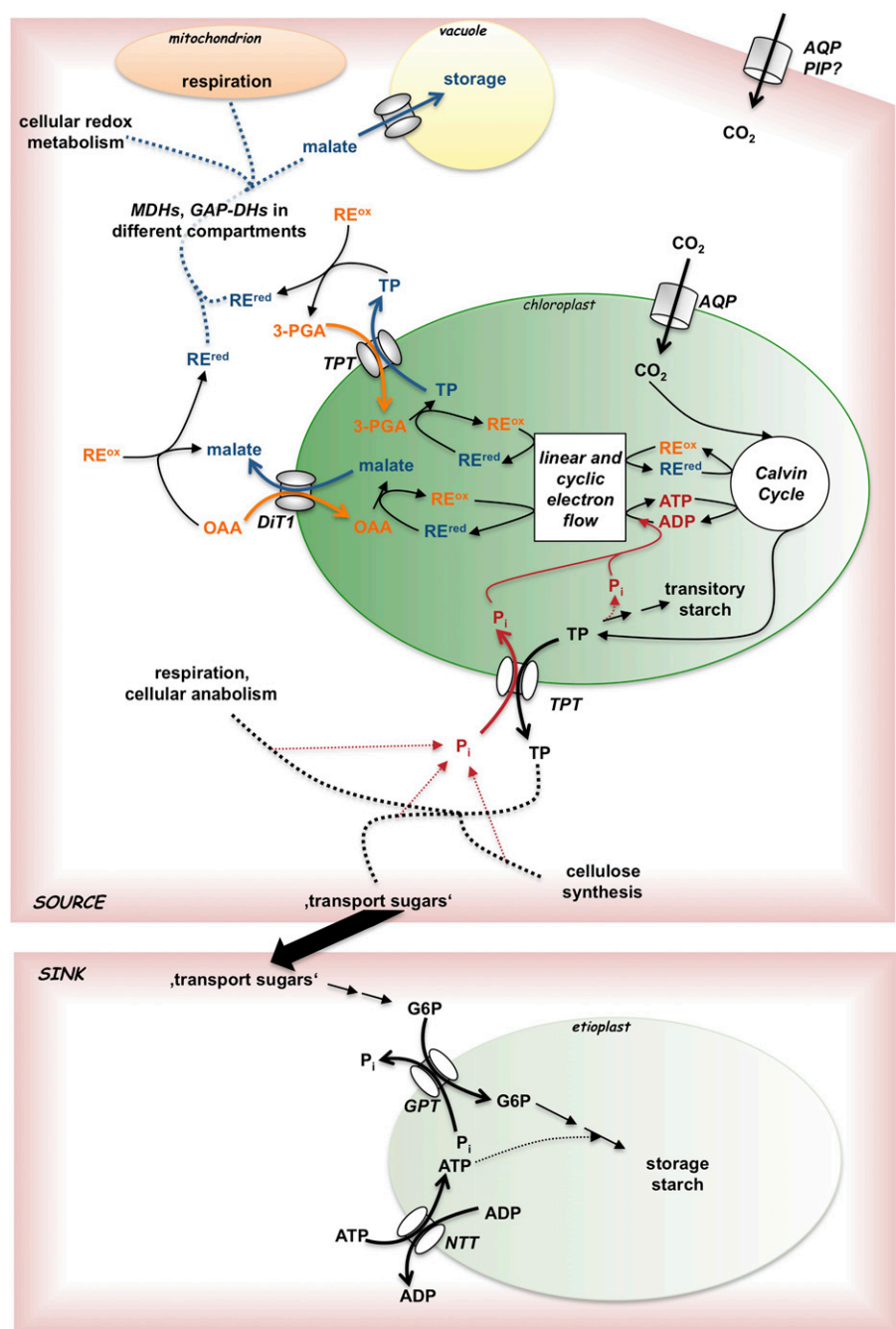
The core reactions of photosynthesis occur exclusively in the chloroplast: (1) the light-driven photosynthetic electron transport chain, which generates reducing equivalents in the form of NADPH and energy equivalents in the form of ATP, and (2) the Calvin-Benson cycle, which uses reducing and energy equivalents to assimilate  $\text{CO}_2$  into triosephosphates (TPs). Organic carbon in the form of TPs represents the principle output of the Calvin-Benson cycle. TPs can either be exported from the chloroplast to the remainder of the cell or they can be metabolized within the chloroplast, for example during transitory starch biosynthesis (Heldt, 1999; Fig. 1, center). Both energy and reducing power generated by the photosynthetic light reactions are also used in the chloroplast for a number of additional anabolic reactions, such as nitrogen and sulfur assimilation, amino acid and lipid biosynthesis, and production of precursors for secondary metabolism. However, the chloroplast is not autonomous—it depends on the remainder of the cell for photosynthesis to function: TPs exported to the cytosol are predominantly converted to transport sugars, such as Suc, and to structural carbohydrates, such as cellulose. Inorganic phosphate ( $\text{Pi}$ ) released from TPs during these biosyntheses is returned to the chloroplast, which is essential for continuous operation of photosynthesis. Indeed, the one-to-one stoichiometry for TP/ $\text{Pi}$  exchange by the TP/phosphate translocator (TPT) provides a regulatory link between photosynthetic rates and cytosolic carbon metabolism. For example, if Suc synthesis in the cytoplasm slows down,  $\text{Pi}$  availability drops and the absence of  $\text{Pi}$  returning to the chloroplast slows photosynthesis. In addition, a toxic by-product of the Rubisco reaction, phosphoglycolate, must be detoxified, excess reducing power

<sup>1</sup> This work was supported by the German Research Council (grant nos. WE 2231/4-1, WE 2231/8-1, SFB TR1, IRTG 1525/1, and FOR 1186 to A.M.P.W.).

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[www.plantphysiol.org/cgi/doi/10.1104/pp.110.164970](http://www.plantphysiol.org/cgi/doi/10.1104/pp.110.164970)

**Figure 1.** Schematic representation of pathways and transport proteins with impact on photosynthetic capacity. RE<sup>red</sup>, Reduced reducing equivalent; RE<sup>ox</sup>, oxidized reducing equivalent; G6P, Glc-6-P; MDH, malate dehydrogenase; GAP-DH, glyceraldehyde phosphate dehydrogenase; for transport protein abbreviations, see legend to Table I. The complex sink source interface is only schematically indicated by an arrow connecting source with sink.



needs to be diffused, and cofactors for the photosynthetic reactions need to be imported from other parts of the cell. Hence, efficient operation of photosynthesis critically depends on the presence of transport proteins that connect the chloroplast with its surroundings.

Chloroplasts, the site of photosynthesis, are surrounded by two membranes, the inner envelope membrane and the outer envelope membrane. The inner envelope is traditionally considered to represent the specificity barrier with a set of highly specific trans-

port proteins, while the outer envelope is considered to be less selective with a set of broad specificity pores.

### THE CORE TRANSPORT PROCESSES INVOLVED IN PHOTOSYNTHESIS

The single most abundant protein in the inner envelope is the TPT, which represents the major pathway for carbon export during the day (Flügge and Heldt, 1984). It functions as an antiporter. That is, it transports TPs in a 1:1 counter exchange with Pi (Flügge and Heldt, 1984).

The net result of each transport step is equivalent to three reduced carbon atoms, with no net transport of phosphate (Table I; Fig. 1). Dissecting the physiological role of this transport protein is complex since TPs can either be exported to the cytosol, or stored inside the chloroplast in the form of transitory starch. If synthesis of Suc that is synthesized from TPs in the cytosol (and/or Suc export to sinks) becomes limited, as indicated by falling cytosolic phosphate concentrations, phosphate becomes unavailable as a counter substrate for TP at the transporter. TP can thus no longer be exported and is rerouted into transitory starch biosynthesis (Flügge and Heldt, 1984). This rerouting provides sufficient metabolic flexibility to allow the plant to survive under laboratory conditions, even if the activity of TPT is compromised by knockdown or knockout (Häusler et al., 2000b, 2000c; Schneider et al., 2002). Transitory starch is broken down during the night predominantly to maltose and to a minor degree to Glc, which are both exported to the cytosol for conversion to Suc that is loaded into the phloem. The amylolytic mobilization of transitory starch does not conserve all of the energy contained in the glycosidic bonds of the starch polymer (Weise et al., 2004) and mobilization and phloem loading are fueled by respiration during the night (Häusler et al., 2000a).

In contrast to photosynthesis in ambient CO<sub>2</sub> concentrations, TPT strongly limits photosynthetic carbon fixation under elevated CO<sub>2</sub> conditions (Häusler et al., 2000c). Under these conditions, both the maximum rate of transitory starch biosynthesis and TP export from the chloroplast colimit the rate of CO<sub>2</sub> assimilation. This indicates that if CO<sub>2</sub> concentrations continue to rise or if photosynthesis is engineered to increase the flux through the pathway, the TPT will become limiting for the rate of CO<sub>2</sub> assimilation.

In contrast to organic carbon, which can be stored if in excess, excess reducing power cannot be stored in the chloroplast and thus must be dissipated. In addition to the chloroplast-intrinsic pathways for dissipation, there exist at least two potential shuttles for the export of reducing power: (1) the TP/3-phosphoglyceric acid (3-PGA) shuttle and (2) the malate/oxaloacetate (OAA) shuttle. The TPT of higher plants is not only capable of exporting reduced carbon but also of reducing power (Flügge and Heldt, 1984). That is, the TPT can exchange TP for 3-PGA, which equals the net movement of one reducing equivalent: TP is exported to the cytosol, oxidized to 3-PGA by cytosolic glyceraldehyde phosphate dehydrogenase, and the resulting 3-PGA is returned to the chloroplast. Analyzing the physiological role of TPT as redox shuttle in knockdown or knockout plants is difficult since the effects of carbon export limitation may confound those of redox export limitation. Introducing a TPT that does not accept 3-PGA and that is therefore unable to act as a reducing equivalent shuttle into a TPT knockout mutant would allow to separately address the two roles of TPT and its possible role as an *in vivo* reducing equivalent shuttle (Linka et al., 2008).

A second possible reducing equivalent shuttle is the dicarboxylate translocator DiT1 (Taniguchi et al., 2002; Renné et al., 2003). It can exchange OAA for malate; hence, it catalyzes the net movement of one reducing equivalent without net C<sub>4</sub> acid transport (Taniguchi et al., 2002; Renné et al., 2003): Malate is exported to the cytosol, where it is oxidized to OAA, which is returned to the chloroplast. Since DiT1, together with DiT2 in a shuttle that involves two transport proteins, also plays a major role in nitrogen assimilation (Weber and Flügge, 2002), similar to TPT these dual roles cannot

**Table I.** List of transport proteins with impact on photosynthetic capacity

Name	Shortcut	Arabidopsis Gene	Substrates	Mode of Transport	Net Transport
Aquaporin	AQP	Analyzed in tobacco ( <i>Nicotiana tabacum</i> )	CO <sub>2</sub>	Channel	CO <sub>2</sub>
Triosephosphate/phosphate translocator	TPT	At5g46110	TP, phosphate	Antiport	Three carbon moieties
			TP, 3-PGA	Antiport	One reducing equivalent, reduced
Dicarboxylate translocator 1	DiT1	At5g12860	OAA, malate	Antiport	One reducing equivalent, reduced
Suc symporter 1	SUT1	Characterized in sugar beet ( <i>Beta vulgaris</i> )	Suc, protons	Symport	12 carbon moieties
Glc-6-P/phosphate translocator	GPT	At5g54800, At1g61800	Glc-6-P, phosphate	Antiport	Six carbon moieties
Adenine nucleotide transporter	NTT	At1g80300, At1g15500	ATP, ADP	Antiport	Energy, phosphate
Dicarboxylate translocator 1	DiT1	At5g12860	2-Oxoglutarate, malate	Antiport	One amino group (together with DiT2)
Dicarboxylate translocator 2	DiT2	At5g64290, At5g64280	Glu, malate	Antiport	One amino group (together with DiT1)
Phosphoenolpyruvate/phosphate translocator	PPT	At5g33320, At3g01550	Phosphoenolpyruvate, phosphate	Antiport	Three carbon moieties
S-adenosyl-Met transporter	SAMT	At4g39460	S-adenosyl-Met, S-adenosyl homo-Cys	Antiport	Activate one carbon group

easily be dissected. Relatively mild repression of DiT1 already limits photosynthesis. This limitation is likely based in part or completely on its role in nitrogen metabolism and not as a redox shuttle (Schneidereit et al., 2006). The reducing equivalents exported by either the TP/3-PGA and/or the OAA/malate shuttle can be recovered and stored in any organelles that have glyceraldehyde phosphate dehydrogenase or malate dehydrogenase activity (Scheibe, 2004), oxidized by the mitochondria (Raghavendra and Padmasree, 2003), or used for redox reactions. Recently, it was also shown that C<sub>4</sub> acids such as malate play an important role as carbon store fueling nocturnal cellular metabolism (Fahnenstich et al., 2007), which requires the vacuole as a storage compartment for malate. Of course, the movement of redox pairs across other organellar membranes and the storage of malate require transport proteins, such as those located in the tonoplast membrane (Emmerlich et al., 2003).

In contrast to both organic carbon and reducing power, ATP transport across the leaf chloroplast envelope likely does not play a major role during the day. The capacity for ATP transport in the chloroplast envelope is 100-fold lower than that for TPs and the major effect of a knockout of the plastidial ATP transporter NTT is on nocturnal, not on photosynthetic metabolism (Reiser et al., 2004).

Transitory starch stored during the day is broken down by the amylolytic pathway during the night and the resulting products maltose and Glc are exported to the cytosol (Weise et al., 2004). The export of maltose is mediated by the maltose exporter MEX1 (Niittylä et al., 2004) and Glc is exported by the plastidic Glc transporter pGlcT (Weber et al., 2000). The nightly mobilization rate of transitory starch is adjusted by the plant so that the starch reservoir lasts until the night ends (Usadel et al., 2008). Inhibition of nightly starch degradation severely inhibits plant growth (Niittylä et al., 2004; Lu and Sharkey, 2006), indicating that sufficient breakdown capacity is critical to optimal use of carbon stored during the day.

In addition to transport proteins that export the products of photosynthesis, the import of substrates also warrants consideration. Until recently, it was assumed that CO<sub>2</sub> entry into the chloroplast occurs by diffusion through the membrane. However, dedicated CO<sub>2</sub> pores belonging to the aquaporin protein family were recently discovered in the plasma membrane and in the chloroplast envelope (Uehlein et al., 2003, 2008). A knockdown of the CO<sub>2</sub> transporting aquaporin resulted in a 15% decrease in the maximal photosynthetic rate, which was most likely due to higher resistance to CO<sub>2</sub> conductance at the chloroplast envelope (Uehlein et al., 2008). The consequence of CO<sub>2</sub> aquaporin overexpression has not yet been reported. However, it is likely that the CO<sub>2</sub> pores are evolutionarily adapted to present day CO<sub>2</sub> concentrations and present day photosynthetic rates in the plants' native environment. Growth in elevated CO<sub>2</sub> concentrations, which we will be facing due to global

change, or in lowered CO<sub>2</sub> conditions such as closed stomates due to drought, may alter flux in a way that requires altered CO<sub>2</sub> conductance.

## TRANSPORT PROCESSES PERIPHERALLY ASSOCIATED WITH PHOTOSYNTHESIS

Sink strength is a major determinant of photosynthetic capacity. Photoassimilates are exported from source cells via the phloem to the sink tissues. At least one transport protein at this interface, the Suc proton symporter SUT1 of the phloem companion cells, controls the photoassimilate transport rate to the sinks (Vaughn et al., 2002). Its abundance and therefore its maximal transport capacity are mediated by Suc in the phloem that in turn depends on sink strength (Vaughn et al., 2002). In sink tissues storage starch is produced from Glc-6-P imported into the amyloplasts. Since amyloplasts are not capable of producing ATP by photophosphorylation, it must be imported from the remainder of the cell. Simultaneous overexpression of the plastidial Glc-6-P/phosphate translocator (GPT) and the ATP transporter NTT in potato (*Solanum tuberosum*) tubers leads to increased sink strength and 19% higher tuber yield. The starch content was increased overall (44%) and on per tuber basis (28%; Zhang et al., 2008). This case clearly demonstrates flux control at the transport protein level, rather than at the level of enzyme activity.

Several other transport proteins apparently limit photosynthesis, although it is not exactly known whether the effect is directly on photosynthetic metabolism or due to pleiotropic effects on cellular metabolism. For example, a mutation in the plastidic phosphoenolpyruvate/phosphate translocator (PPT) causes a reticulate leaf phenotype and affects the electron transfer rates but not overall carbon assimilation rates (Streatfield et al., 1999). PPT imports one of the substrates of the shikimic acid pathway, phosphoenolpyruvate, from the cytosol. The shikimate pathway provides one of the substrates for plastoquinone biosynthesis, which might explain the observed effects on electron transfer rates (Voll et al., 2003). A mutation in the plastidic S-adenosyl-Met transporter SAMT, affects prenyllipid synthesis and therefore the synthesis of a range of cofactors essential to photosynthesis, among them chlorophyll and plastoquinone. Consequentially, knockdowns in SAMT appear pale and have retarded growth, indicating less net carbon gain (Bouvier et al., 2006). These examples show that it is not always straightforward to dissect direct effects on photosynthetic metabolism from pleiotropic metabolic effects that indirectly feed back onto photosynthesis.

Although the core reactions of photosynthesis occur exclusively in the chloroplast, photorespiration, the recycling of carbon lost through the oxygenation reaction of Rubisco, requires peroxisomes and mitochondria (Bauwe et al., 2010). Since up to one-third of inorganic carbon fixed during the day is lost again through

photorespiration in  $C_3$  plants, the flux through this pathway is very high, in mature leaves probably second only to photosynthesis itself (Bauwe et al., 2010). The enzymatic reactions of photorespiration have been mostly resolved at the molecular level, however, all transport proteins, except for those involved in nitrogen reassimilation, are unknown (Bauwe et al., 2010). It is thus difficult to estimate whether transport proteins exert control over the flux through this pathway or whether transport proteins are present in excess, such as the enzymes involved in the pathway (Zhu et al., 2007). Although metabolic channeling through organellar extensions, such as stromules and matrixules has been suggested, it is more likely that controlled metabolite exchange by transport proteins ensures flux through the pathway (Foyer and Noctor, 2007). Physical proximity of peroxisomes and mitochondria to chloroplasts, as it is known from electron micrographs, is apparently crucial for efficient photorespiration. A mutation in the peroxisomal PEX10 protein causes loss of physical association and a mild photorespiratory phenotype, indicating that short pathways for diffusion between organelles are important for maintaining high flux through the pathway (Schumann et al., 2007). The role of peroxisomes in photosynthetic metabolism beyond photorespiration has not yet been fully resolved.

Photorespiration aside, mitochondrial metabolism is vital for chloroplast function and photosynthetic capacity. Mitochondrial inhibitors like oligomycin, sodium azide, or antimycin A and transgenic modifications of the mitochondrial electron transport chain have marked influence on photosynthetic capacity (Raghavendra and Padmasree, 2003; Noctor et al., 2007). At least one transport protein is known to be involved in this intricate balance, the uncoupling protein UCP1. A UCP1 mutant in *Arabidopsis thaliana* is characterized by lower  $CO_2$  assimilation rates, although its stomatal conductance was not altered. It was suggested that lack of uncoupling protein function adversely affects mitochondrial redox poise, which in turn impacts photosynthesis (Sweetlove et al., 2006). Thus, photosynthesis, although confined to the chloroplast, is part of an intricate cross-compartment network that is well connected throughout the cell and dependent on its connections. Attempts to increase flux through photosynthesis thus likely require adjustments to the transport capacity of the chloroplast membrane and of other organellar membranes as well.

#### **$C_4$ PHOTOSYNTHESIS: NATURE'S SUCCESSFUL SOLUTION TO SUPERCHARGING PHOTOSYNTHESIS**

$C_4$  photosynthesis is highly efficient due to the reduction of carbon loss by photorespiration. This is achieved, at the expense of additional ATPs per  $CO_2$  fixed, through increasing the  $CO_2$  concentration in the vicinity of Rubisco, thereby suppressing the oxygenation reaction of Rubisco to less than 1% of that observed in  $C_3$  plants (von Caemmerer and Furbank,

2003). The  $C_4$  photosynthetic reactions occur in different cell types, mesophyll and bundle sheath cells, and involve at least two distinct chloroplast types, one in each cell type (Hatch, 1987). The flux through the  $C_4$  pathway is higher than the apparent rate of  $CO_2$  assimilation and likely represents one of the highest metabolite fluxes known in plants (Weber and von Caemmerer, 2010). Comparative quantitative proteomics as well as transcriptomics clearly demonstrated that the high metabolite flux needed to sustain  $C_4$  photosynthesis is achieved by strongly increased transport protein abundance (Bräutigam et al., 2008, 2011; Friso et al., 2010). Hence, during evolution of the  $C_4$  photosynthetic pathway, transport capacity became limiting and the amounts of transport proteins were increased to cope with the increased demand on flux.  $C_4$  photosynthesis thus serves as a prime example demonstrating the importance of transport proteins for achieving high rates of photosynthetic carbon assimilation.

#### **CONCLUDING REMARKS**

We set out to address the question—do metabolite transport processes limit photosynthesis? Unfortunately, the question cannot be answered with a simple yes or no. Plants have evolved somewhat elastic transport capacities to cope with environmental variations, such as shading or high light intensities. However, this innate flexibility is rather limited, as has been demonstrated by knockdown and knockout plant lines showing reduced capacity of TP transport by TPT. Global change, leading to increasing  $CO_2$  concentrations in the atmosphere, as well as attempts at increasing photosynthetic rates by engineering of the photosynthetic pathway may easily overburden the extant capacity of the transport systems both in and outside of the chloroplast. While altering transport capacity alone is unlikely to change photosynthetic capacity, altering photosynthetic capacity by other means may quickly render transport capacity as a limiting factor, as demonstrated by the strong control exerted by TPT over the maximal rate of photosynthesis at elevated  $CO_2$  concentrations. A further case in point is  $C_4$  photosynthesis, in which increased amounts and activities of enzymes of the  $C_4$  pathway are accompanied by strongly increased amounts of the required transporter proteins.

#### **ACKNOWLEDGMENTS**

We regret omission of many relevant citations due to space constraints.

Received August 30, 2010; accepted September 19, 2010; published September 20, 2010.

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